

What is claimed is:

1. A method to produce glucosamine or N-acetylglucosamine by fermentation, comprising:

5

a) culturing in a fermentation medium a microorganism which comprises at least one genetic modification that increases the activity of glucosamine-6-phosphate acetyltransferase; and

b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, and N-acetylglucosamine.

5

2. The method of Claim 1, wherein the genetic modification to increase the activity of glucosamine-6-phosphate acetyltransferase provides a result selected from the group consisting of: increased enzymatic activity of glucosamine-6-phosphate acetyltransferase; overexpression of glucosamine-6-phosphate acetyltransferase by the microorganism; reduced N-acetylglucosamine-6-phosphate product inhibition of the glucosamine-6-phosphate acetyltransferase; and increased affinity of glucosamine-6-phosphate acetyltransferase for glucosamine-6-phosphate.

3. The method of Claim 1, wherein the microorganism is transformed with at least one recombinant nucleic acid molecule comprising a nucleic acid sequence encoding the glucosamine-6-phosphate acetyltransferase.

4. The method of Claim 3, wherein the nucleic acid sequence encoding a glucosamine-6-phosphate acetyltransferase has at least one genetic modification which increases the enzymatic activity of the glucosamine-6-phosphate acetyltransferase.

5. The method of Claim 3, wherein the glucosamine-6-phosphate acetyltransferase has an amino acid sequence that is at least about 35% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:30, SEQ ID NO:32 and SEQ ID NO:34, wherein the glucosamine-6-phosphate acetyltransferase has enzymatic activity.

5

6. The method of Claim 3, wherein the glucosamine-6-phosphate acetyltransferase has an amino acid sequence that is at least about 50% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:30, SEQ ID NO:32 and SEQ ID NO:34, wherein the glucosamine-6-phosphate acetyltransferase has enzymatic activity.

7. The method of Claim 3, wherein the glucosamine-6-phosphate acetyltransferase has an amino acid sequence that is at least about 70% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:30, SEQ ID NO:32 and SEQ ID NO:34, wherein the glucosamine-6-phosphate acetyltransferase has enzymatic activity.

8. The method of Claim 3, wherein the glucosamine-6-phosphate acetyltransferase has an amino acid sequence selected from the group consisting of SEQ ID NO:30, SEQ ID NO:32 and SEQ ID NO:34.

9. The method of Claim 3, wherein expression of the recombinant nucleic acid molecule is inducible.

10. The method of Claim 9, wherein expression of the recombinant nucleic acid molecule is inducible by lactose.

11. The method of Claim 10, wherein the microorganism further comprises a genetic modification to reduce inhibition of transcription induction by lactose.

12. The method of Claim 11, wherein the genetic modification comprises a partial or complete deletion or inactivation of a gene encoding a LacI repressor protein.

13. The method of Claim 1, wherein the microorganism further comprises at least one genetic modification that increases the activity of glucosamine-6-phosphate synthase.

14. The method of Claim 13, wherein the microorganism is transformed with at least one recombinant nucleic acid molecule comprising a nucleic acid sequence encoding the glucosamine-6-phosphate synthase.

15. The method of Claim 14, wherein the glucosamine-6-phosphate synthase comprises an amino acid sequence that is at least about 35% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID

NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ
5 ID NO:18, and SEQ ID NO:20, wherein the glucosamine-6-phosphate synthase has
enzymatic activity.

16. The method of Claim 14, wherein the glucosamine-6-phosphate synthase
comprises an amino acid sequence that is at least about 50% identical to an amino acid
sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID
NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ
5 ID NO:18, and SEQ ID NO:20, wherein the glucosamine-6-phosphate synthase has
enzymatic activity.

17. The method of Claim 14, wherein the glucosamine-6-phosphate synthase
comprises an amino acid sequence that is at least about 70% identical to an amino acid
sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID
NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ
5 ID NO:18, and SEQ ID NO:20, wherein the glucosamine-6-phosphate synthase has
enzymatic activity.

18. The method of Claim 14, wherein the glucosamine-6-phosphate synthase
comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:2,
SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID
NO:14, SEQ ID NO:16, SEQ ID NO:18, and SEQ ID NO:20.

19. The method of Claim 14, wherein the glucosamine-6-phosphate synthase has
a modification to reduce product inhibition of the glucosamine-6-phosphate synthase as
compared to the wild-type glucosamine-6-phosphate synthase.

20. The method of Claim 19, wherein the glucosamine-6-phosphate synthase
comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:4,
SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14.

21. The method of Claim 1, wherein the microorganism further comprises at least
one genetic modification that decreases the activity of glucosamine-6-phosphate deaminase.

22. The method of Claim 21, wherein the genetic modification to decrease the
activity of glucosamine-6-phosphate deaminase comprises a partial or complete deletion or

inactivation of an endogenous gene encoding the glucosamine-6-phosphate deaminase in the microorganism.

23. The method of Claim 13, wherein the microorganism further comprises at least one genetic modification that decreases the activity of glucosamine-6-phosphate deaminase.

24. The method of Claim 23, wherein the genetic modification to decrease the activity of glucosamine-6-phosphate deaminase comprises a partial or complete deletion or inactivation of an endogenous gene encoding the glucosamine-6-phosphate deaminase in the microorganism.

25. The method of Claim 1, wherein the step of culturing includes the step of maintaining the carbon source at a concentration of from about 0.5% to about 5% in the fermentation medium.

26. The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising yeast extract.

27. The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising a carbon source selected from the group consisting of glucose, fructose, a pentose sugar, lactose and gluconic acid.

28. The method of Claim 27, wherein the pentose sugar is selected from the group consisting of ribose, xylose, and arabinose.

29. The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising glucose and ribose.

30. The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising glucose and gluconic acid.

31. The method of Claim 1, wherein the step of culturing is performed at a temperature of from about 25°C to about 45°C.

32. The method of Claim 1, wherein the step of culturing is performed at about 37°C.

33. The method of Claim 1, wherein the step of culturing is performed at a pH of from about pH 4 to about pH 7.5.

34. The method of Claim 1, wherein the step of culturing is performed at a pH of from about pH 6.7 to about pH 7.5.

35. The method of Claim 1, wherein the step of culturing is performed at a pH of from about pH 4.5 to about pH 5.

36. The method of Claim 1, wherein the microorganism is selected from the group consisting of bacteria and fungi.

37. The method of Claim 1, wherein the microorganism is selected from the group consisting of bacteria and yeast.

38. The method of Claim 1, wherein the microorganism is a bacterium from a genus selected from the group consisting of: *Escherichia*, *Bacillus*, *Lactobacillus*, *Pseudomonas* and *Streptomyces*.

39. The method of Claim 1, wherein the microorganism is a bacterium from a species selected from the group consisting *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Lactobacillus brevis*, *Pseudomonas aeruginosa* and *Streptomyces lividans*.

40. The method of Claim 1, wherein microorganism is a yeast from a genus selected from the group consisting of: *Saccharomyces*, *Candida*, *Hansenula*, *Pichia*, *Kluveromyces*, and *Phaffia*.

41. The method of Claim 1, wherein microorganism is a yeast from a species selected from the group consisting of: *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans*, *Hansenula polymorpha*, *Pichia pastoris*, *P. canadensis*, *Kluyveromyces marxianus* and *Phaffia rhodozyma*.

42. The method of Claim 1, wherein the microorganism is a fungus from a genus selected from the group consisting of: *Aspergillus*, *Absidia*, *Rhizopus*, *Chrysosporium*, *Neurospora* and *Trichoderma*.

43. The method of Claim 1, wherein the microorganism is a fungus from a species selected from the group consisting of: *Aspergillus niger*, *A. nidulans*, *Absidia coerulea*, *Rhizopus oryzae*, *Chrysosporium lucknowense*, *Neurospora crassa*, *N. intermedia* and *Trichoderm reesei*.

44. The method of Claim 1, wherein the microorganism further comprises a genetic modification to increase phosphoglucoisomerase activity in the microorganism.

45. The method of Claim 44, wherein the microorganism is transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding the phosphoglucoisomerase.

46. The method of Claim 44, wherein the phosphoglucoisomerase comprises an amino acid sequence of SEQ ID NO:105.

47. The method of Claim 1, wherein the microorganism further comprises a partial or complete deletion or inactivation of phosphofructokinase in the microorganism.

48. The method of Claim 1, wherein the microorganism further comprises a genetic modification to increase the activity of glutamine synthetase.

49. The method of Claim 48, wherein the microorganism has been transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding the glutamine synthetase.

50. The method of Claim 48, wherein the glutamine synthetase comprises an amino acid sequence of SEQ ID NO:89.

51. The method of Claim 1, wherein the microorganism further comprises a genetic modification to increase the activity of glucose-6-phosphate dehydrogenase.

52. The method of Claim 51, wherein the microorganism has been transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding the glucose-6-phosphate dehydrogenase.

53. The method of Claim 51, wherein the glucose-6-phosphate dehydrogenase comprises an amino acid sequence of SEQ ID NO:95.

54. The method of Claim 1, wherein the microorganism further comprises a partial or complete deletion or inactivation of genes encoding enzymes responsible for glycogen synthesis in the microorganism.

55. The method of Claim 54, wherein the genes encoding enzymes responsible for glycogen synthesis comprise ADP-glucose pyrophosphorylase, glycogen synthase and a branching enzyme.

56. The method of Claim 1, wherein the genetic modifications do not inhibit the ability of the microorganism to metabolize galactose.

57. The method of Claim 1, wherein the step of collecting comprises recovering an intracellular product from the microorganism selected from the group consisting of: intracellular glucosamine-6-phosphate, glucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine and glucosamine or recovering an extracellular product from the fermentation medium selected from the group consisting of: glucosamine and N-acetylglucosamine.

58. The method of Claim 1, further comprising a step selected from the group consisting of:

- a) purifying a product selected from the group consisting of glucosamine and N-acetylglucosamine from the fermentation medium;
- b) recovering a product selected from the group consisting of glucosamine-6-phosphate, glucosamine-1-phosphate, N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate from the microorganism;
- c) dephosphorylating a product selected from the group consisting of glucosamine-6-phosphate and glucosamine-1-phosphate to produce glucosamine; and
- d) dephosphorylating a product selected from the group consisting of N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate to produce N-acetylglucosamine
- e) treating a product selected from the group consisting of N-acetylglucosamine, N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate to produce a glucosamine product selected from the group consisting of: glucosamine, glucosamine-6-phosphate and glucosamine-1-phosphate.

15 59. The method of Claim 54, wherein step (e) comprises hydrolyzing the product selected from the group consisting of N-acetylglucosamine, N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate, under acid and heat conditions or by enzymatic deacetylation.

60. The method of Claim 1, wherein N-acetylglucosamine produced by the fermentation method is recovered by precipitating N-acetylglucosamine-containing solids from the fermentation broth.

61. The method of Claim 1, wherein N-acetylglucosamine produced by the fermentation method is recovered by crystallizing N-acetylglucosamine-containing solids from the fermentation broth.

62. A method to produce glucosamine or N-acetylglucosamine by fermentation, comprising:

- a) culturing in a fermentation medium a microorganism which comprises at least one genetic modification that increases the activity of glucosamine-6-phosphate deaminase; and
- b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, and N-acetylglucosamine.

63. The method of Claim 62, wherein the genetic modification provides a results selected from the group consisting of: overexpression of glucosamine-6-phosphate deaminase by the microorganism, increased enzymatic activity of glucosamine-6-phosphate deaminase, increased reverse reaction of glucosamine-6-phosphate deaminase to form increased glucosamine-6-phosphate, reduced forward reaction of glucosamine-6-phosphate deaminase to form reduced fructose-6-phosphate, increased affinity of glucosamine-6-phosphate deaminase for fructose-6-phosphate, reduced affinity of glucosamine-6-phosphate deaminase for glucosamine-6-phosphate, and reduced glucosamine-6-phosphate product inhibition of the glucosamine-6-phosphate deaminase.

64. The method of Claim 62, wherein the microorganism is transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a glucosamine-6-phosphate deaminase.

65. The method of Claim 64, wherein the nucleic acid sequence encoding a glucosamine-6-phosphate deaminase has at least one genetic modification which increases the enzymatic activity of the glucosamine-6-phosphate deaminase.

66. The method of Claim 64, wherein the glucosamine-6-phosphate deaminase has an amino acid sequence that is at least about 35% identical to an amino acid sequence of SEQ ID NO:42, wherein the glucosamine-6-phosphate deaminase has enzymatic activity.

67. The method of Claim 64, wherein the glucosamine-6-phosphate deaminase has an amino acid sequence of SEQ ID NO:42.

68. The method of Claim 62, wherein the microorganism further comprises a genetic modification to decrease the activity of glucosamine-6-phosphate synthase.

69. The method of Claim 68, wherein the genetic modification to decrease the activity of glucosamine-6-phosphate synthase is a partial or complete deletion or inactivation of an endogenous gene encoding glucosamine-6-phosphate synthase in the microorganism.

70. The method of Claim 62, wherein the microorganism further comprises a genetic modification to increase the activity of glucosamine-6-phosphate N-acetyltransferase.

71. The method of Claim 70, wherein the genetic modification provides a result selected from the group consisting of: increased enzymatic activity of glucosamine-6-phosphate acetyltransferase; overexpression of glucosamine-6-phosphate acetyltransferase by the microorganism; reduced N-acetylglucosamine-6-phosphate product inhibition of the glucosamine-6-phosphate acetyltransferase; and increased affinity of glucosamine-6-phosphate acetyltransferase for glucosamine-6-phosphate.

72. The method of Claim 70, wherein the microorganism is transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding the glucosamine-6-phosphate N-acetyltransferase.

73. The method of Claim 70, wherein the glucosamine-6-phosphate N-acetyltransferase comprises an amino acid sequence that is at least about 35% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:30, SEQ ID NO:32 and SEQ ID NO:34, wherein the glucosamine-6-phosphate acetyltransferase has enzymatic activity.

74. The method of Claim 70, wherein the glucosamine-6-phosphate N-acetyltransferase comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:30, SEQ ID NO:32 and SEQ ID NO:34.

75. The method of Claim 70, wherein the microorganism further comprises a genetic modification to decrease the activity of glucosamine-6-phosphate synthase.

76. The method of Claim 75, wherein the genetic modification to decrease the activity of glucosamine-6-phosphate synthase is a partial or complete deletion or inactivation of an endogenous gene encoding glucosamine-6-phosphate synthase in the microorganism.

77. The method of Claim 62, wherein the microorganism further comprises a genetic modification to increase the activity of glucosamine-1-phosphate N-acetyltransferase.

78. The method of Claim 77, wherein the genetic modification provides a result selected from the group consisting of: increased enzymatic activity of glucosamine-1-phosphate N-acetyltransferase; reduced N-acetylglucosamine-1-phosphate uridyltransferase enzymatic activity; overexpression of an enzyme having glucosamine-1-phosphate N-acetyltransferase activity by the microorganism; increased affinity of glucosamine-1-phosphate N-acetyltransferase for glucosamine-1-phosphate; reduced affinity of an glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase for N-acetylglucosamine-1-phosphate; and reduced N-acetylglucosamine-1-phosphate product inhibition of the glucosamine-1-phosphate N-acetyltransferase.

79. The method of Claim 77, wherein the microorganism comprises a bifunctional glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase, wherein the glucosamine-1-phosphate N-acetyltransferase activity is increased.

80. The method of Claim 77, wherein the microorganism is transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase or a nucleic acid sequence encoding a glucosamine-1-phosphate N-acetyltransferase.

81. The method of Claim 80, wherein the nucleic acid sequence encoding a glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase or a glucosamine-1-phosphate N-acetyltransferase has at least one genetic modification which increases the activity of the glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase or the glucosamine-1-phosphate N-acetyltransferase, respectively.

82. The method of Claim 80, wherein the glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase has an amino acid sequence that is at least about 35% identical to an amino acid sequence of SEQ ID NO:56,

wherein the glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase has glucosamine-1-phosphate N-acetyltransferase enzymatic activity.

83. The method of Claim 80, wherein the glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase has an amino acid sequence of SEQ ID NO:56.

84. The method of Claim 80, wherein the nucleic acid sequence encodes a truncated glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase having glucosamine-1-phosphate N-acetyltransferase activity, and reduced or no N-acetylglucosamine-1-phosphate uridyltransferase activity.

85. The method of Claim 84, wherein the truncated glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase has an amino acid sequence that is at least about 35% identical to an amino acid sequence of SEQ ID NO:58, wherein the truncated glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase has glucosamine-1-phosphate N-acetyltransferase enzymatic activity.

86. The method of Claim 84, wherein the truncated glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase has an amino acid sequence of SEQ ID NO:58.

87. The method of Claim 77, wherein the microorganism further comprises a genetic modification to decrease the activity of glucosamine-6-phosphate synthase.

88. The method of Claim 87, wherein the genetic modification to decrease the activity of glucosamine-6-phosphate synthase is a partial or complete deletion or inactivation of an endogenous gene encoding glucosamine-6-phosphate synthase in the microorganism.

89. The method of Claim 62, wherein the step of collecting comprises recovering an intracellular product from the microorganism selected from the group consisting of: intracellular glucosamine-6-phosphate, glucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine and glucosamine or recovering an extracellular product from the fermentation medium selected from the group consisting of: glucosamine and N-acetylglucosamine.

90. A method to produce glucosamine or N-acetylglucosamine by fermentation, comprising:

- a) culturing in a fermentation medium a microorganism which comprises at least one genetic modification that decreases the activity of glucosamine-6-phosphate deaminase and at least one genetic modification that increases the activity of glucosamine-1-phosphate N-acetyltransferase; and
- b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, and N-acetylglucosamine.

10 91. The method of Claim 90, wherein the genetic modification to decrease the activity of glucosamine-6-phosphate deaminase comprises a partial or complete deletion or inactivation of an endogenous gene encoding the glucosamine-6-phosphate deaminase in the microorganism.

92. The method of Claim 90, wherein the genetic modification to increase the activity of glucosamine-1-phosphate N-acetyltransferase provides a result selected from the group consisting of: increased enzymatic activity of glucosamine-1-phosphate N-acetyltransferase; reduced N-acetylglucosamine-1-phosphate uridyltransferase enzymatic activity; overexpression of an enzyme having glucosamine-1-phosphate N-acetyltransferase activity by the microorganism; increased affinity of glucosamine-1-phosphate N-acetyltransferase for glucosamine-1-phosphate; reduced affinity of an glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase for N-acetylglucosamine-1-phosphate; and reduced N-acetylglucosamine-1-phosphate product inhibition of the glucosamine-1-phosphate N-acetyltransferase.

10 93. The method of Claim 90, wherein the microorganism comprises a bifunctional glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase, wherein the glucosamine-1-phosphate N-acetyltransferase activity is increased.

94. The method of Claim 90, wherein the microorganism is transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase or a nucleic acid sequence encoding a glucosamine-1-phosphate N-acetyltransferase.

5
95. The method of Claim 94, wherein the nucleic acid sequence encoding a glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase or a glucosamine-1-phosphate N-acetyltransferase has at least one genetic modification which increases the activity of the glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase or the glucosamine-1-phosphate N-acetyltransferase, respectively.

96. The method of Claim 94, wherein the glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase has an amino acid sequence that is at least about 35% identical to an amino acid sequence of SEQ ID NO:56, wherein the glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase has glucosamine-1-phosphate N-acetyltransferase enzymatic activity.

97. The method of Claim 94, wherein the glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase has an amino acid sequence of SEQ ID NO:56.

98. The method of Claim 94, wherein the nucleic acid sequence encodes a truncated glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase having glucosamine-1-phosphate N-acetyltransferase activity, and reduced or no N-acetylglucosamine-1-phosphate uridyltransferase activity.

99. The method of Claim 98, wherein the truncated glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase has an amino acid sequence that is at least about 35% identical to an amino acid sequence of SEQ ID NO:58, wherein the truncated glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase has glucosamine-1-phosphate N-acetyltransferase enzymatic activity.

100. The method of Claim 98, wherein the truncated glucosamine-1-phosphate N-acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase has an amino acid sequence of SEQ ID NO:58.

101. The method of Claim 90, wherein the microorganism further comprises at least one genetic modification that increases the activity of glucosamine-6-phosphate synthase.

102. The method of Claim 101, wherein the genetic modification to increase the activity of glucosamine-6-phosphate synthase comprises transformation of the microorganism with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding the glucosamine-6-phosphate synthase.

103. The method of Claim 101, wherein the glucosamine-6-phosphate synthase comprises an amino acid sequence that is at least about 35% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, and SEQ ID NO:20, wherein the glucosamine-6-phosphate synthase has enzymatic activity.

104. The method of Claim 101, wherein the glucosamine-6-phosphate synthase comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, and SEQ ID NO:20.

105. The method of Claim 101, wherein the glucosamine-6-phosphate synthase has a modification to reduce product inhibition of the glucosamine-6-phosphate as compared to the wild-type glucosamine-6-phosphate synthase.

106. The method of Claim 105, wherein the glucosamine-6-phosphate synthase comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14.

107. The method of Claim 90, wherein the step of collecting comprises recovering an intracellular product from the microorganism selected from the group consisting of: intracellular glucosamine-6-phosphate, glucosamine-1-phosphate, N-acetylglucosamine-6-

phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine and glucosamine or
5 recovering an extracellular product from the fermentation medium selected from the group
consisting of: glucosamine and N-acetylglucosamine.

108. A method to produce glucosamine or N-acetylglucosamine by fermentation, comprising:

- a) culturing in a fermentation medium a microorganism which comprises an endogenous glucosamine-6-phosphate acetyltransferase and at least one genetic modification to increase the activity of glucosamine-6-phosphate synthase; and
- b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, and N-acetylglucosamine.

109. The method of Claim 108, wherein the microorganism is transformed with at least one recombinant nucleic acid molecule comprising a nucleic acid sequence encoding the glucosamine-6-phosphate synthase.

110. The method of Claim 109, wherein the glucosamine-6-phosphate synthase comprises an amino acid sequence that is at least about 35% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, and SEQ ID NO:20, wherein the glucosamine-6-phosphate synthase has enzymatic activity.

111. The method of Claim 109, wherein the glucosamine-6-phosphate synthase comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, and SEQ ID NO:20.

112. The method of Claim 109, wherein the glucosamine-6-phosphate synthase has a modification to reduce product inhibition of the glucosamine-6-phosphate synthase as compared to the wild-type glucosamine-6-phosphate synthase.

113. The method of Claim 112, wherein the glucosamine-6-phosphate synthase comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14.

114. The method of Claim 108, wherein the microorganism further comprises at least one genetic modification that decreases the activity of glucosamine-6-phosphate deaminase.

115. The method of Claim 108, wherein the method is a method to produce N-acetylglucosamine by fermentation, and wherein the step of collecting comprises collecting a product produced from the step of culturing which is selected from the group consisting of N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, and N-acetylglucosamine.

5

116. A genetically modified microorganism comprising at least one genetic modification that increases the activity of glucosamine-6-phosphate acetyltransferase.

117. The genetically modified microorganism of Claim 116, wherein the microorganism further comprises at least one genetic modification that increases the activity of glucosamine-6-phosphate synthase.

118. The genetically modified microorganism of Claim 116, wherein the microorganism further comprises at least one genetic modification that decreases the activity of glucosamine-6-phosphate deaminase.

119. The genetically modified microorganism of Claim 116, wherein the microorganism further comprises at least one genetic modification that increases the activity of glucosamine-6-phosphate synthase.

120. A genetically modified microorganism comprising at least one genetic modification that increases the activity of glucosamine-6-phosphate deaminase.

121. The genetically modified microorganism of Claim 120, wherein the microorganism further comprises a genetic modification to decrease the activity of glucosamine-6-phosphate synthase.

122. The genetically modified microorganism of Claim 120, wherein the microorganism further comprises a genetic modification to increase the activity of glucosamine-6-phosphate N-acetyltransferase.

123. The genetically modified microorganism of Claim 122, wherein the microorganism further comprises a genetic modification to decrease the activity of glucosamine-6-phosphate synthase.

124. The genetically modified microorganism of Claim 120, wherein the microorganism further comprises a genetic modification to increase the activity of glucosamine-1-phosphate N-acetyltransferase.

125. The genetically modified microorganism of Claim 124, wherein the microorganism further comprises a genetic modification to decrease the activity of glucosamine-6-phosphate synthase.

126. A genetically modified microorganism comprising at least one genetic modification that decreases the activity of glucosamine-6-phosphate deaminase and at least one genetic modification that increases the activity of glucosamine-1-phosphate N-acetyltransferase.

127. The genetically modified microorganism of Claim 126, wherein the microorganism further comprises at least one genetic modification that increases the activity of glucosamine-6-phosphate synthase.

128. A method to produce N-acetylglucosamine, comprising:
- a) obtaining a fermentation broth containing solubilized N-acetylglucosamine that is a product of a fermentation process; and
 - b) recovering N-acetylglucosamine-containing solids from the fermentation broth.
129. The method of Claim 128, further comprising removing cellular material from the fermentation broth.
130. The method of Claim 128, further comprising decolorizing the fermentation broth.
131. The method of Claim 130, wherein the step of decolorizing is selected from the group consisting of multiple N-acetylglucosamine crystallizations, activated carbon treatment, and chromatographic decolorization.
132. The method of Claim 128, further comprising the step of contacting the fermentation broth with an ion exchange resin.
133. The method of Claim 132, wherein the step of contacting the fermentation broth with an ion exchange resin comprises contacting the fermentation broth with an anion exchange resin and a cation exchange resin.
134. The method of Claim 133, wherein the step of contacting the fermentation broth with an anion exchange resin and a cation exchange resin comprises contacting the fermentation broth with a mixed bed of anion and cation exchange resins.
135. The method of Claim 128, wherein the step recovering comprises precipitating N-acetylglucosamine-containing solids from the fermentation broth.
136. The method of Claim 128, wherein the step recovering comprises crystallizing N-acetylglucosamine-containing solids from the fermentation broth.
137. The method of Claim 128, wherein the step of recovering comprises concentrating the fermentation broth containing solubilized N-acetylglucosamine.
138. The method of Claim 137, wherein the step of concentrating is conducted at less than atmospheric pressure.

139. The method of Claim 137, wherein the step of concentrating is conducted by membrane separation.

140. The method of Claim 137, wherein the step of concentrating is conducted at a temperature of between about 40°C and about 75°C.

141. The method of Claim 137, wherein the step of concentrating is conducted at a temperature of between about 45°C and about 55°C.

142. The method of Claim 137, wherein the step of concentrating is conducted to achieve a solids content in the fermentation broth of at least about 30% solids.

143. The method of Claim 137, wherein the step of concentrating is conducted to achieve a solids content in the fermentation broth of at least about 40% solids.

144. The method of Claim 137, wherein the step of concentrating is conducted to achieve a solids content in the fermentation broth of at least about 45% solids.

145. The method of Claim 137, further comprising cooling the fermentation broth after the step of concentrating.

146. The method of Claim 145, the fermentation broth is cooled to between about -5°C and about 45°C.

147. The method of Claim 145, the fermentation broth is cooled to between about -5°C and about room temperature.

148. The method of Claim 145, the fermentation broth is cooled to about room temperature.

149. The method of Claim 145, further comprising seeding the fermentation broth with crystals of N-acetylglucosamine.

150. The method of Claim 149, wherein the seed crystals of N-acetylglucosamine are selected from the group consisting of N-acetylglucosamine crystals formed by nucleation in the fermentation broth and externally provided N-acetylglucosamine crystals.

151. The method of Claim 128, wherein the step of recovering comprises contacting N-acetylglucosamine with a water miscible solvent.

152. The method of Claim 151, wherein the water miscible solvent is selected from the group consisting of isopropyl alcohol (IPA), ethanol, methanol, acetone, tetrahydrofuran, dimethylsulfoxide, dimethylformamide, dioxane and acetonitrile.

153. The method of Claim 128, further comprising drying the recovered N-acetylglucosamine-containing solids.

154. The method of Claim 153, further comprising washing the dried N-acetylglucosamine-containing solids with a water miscible solvent.

155. The method of Claim 128, further comprising dissolving the recovered N-acetylglucosamine-containing solids to form an N-acetylglucosamine solution and recovering N-acetylglucosamine-containing solids from the solution.

156. The method of Claim 128, further filtration of the fermentation broth to remove bacterial endotoxins.

157. A method to produce glucosamine from a source of N-acetylglucosamine, comprising:

- a) obtaining a source of N-acetylglucosamine selected from the group consisting of: N-acetylglucosamine, N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate; and,
- b) treating the source of N-acetylglucosamine of (a) to produce a glucosamine product selected from the group consisting of: glucosamine, glucosamine-6-phosphate and glucosamine-1-phosphate, from the source of N-acetylglucosamine.

158. The method of Claim 157, wherein the source of N-acetylglucosamine is at least about 40% N-acetylglucosamine as a percentage of dry solids in the source.

159. The method of Claim 157, wherein the source of N-acetylglucosamine is N-acetylglucosamine that has been produced by a fermentation process.

160. The method of Claim 157, wherein the source of N-acetylglucosamine is a fermentation broth containing N-acetylglucosamine that was produced by a fermentation process, wherein the fermentation broth has been treated to substantially remove cellular material.

161. The method of Claim 157, wherein the source of N-acetylglucosamine is provided as a solid or in a solution.

162. The method of Claim 161, wherein the source of N-acetylglucosamine is suspended in an aqueous, low-boiling, primary or secondary alcohol.

163. The method of Claim 157, wherein step (b) of treating comprises hydrolyzing the source of N-acetylglucosamine under acid and heat conditions.

164. The method of Claim 163, wherein the step of hydrolyzing is performed at a temperature of from about 60°C to about 100°C.

165. The method of Claim 163, wherein the step of hydrolyzing is performed at a temperature of from about 70°C to about 90°C.

166. The method of Claim 163, wherein the step of hydrolyzing is performed using a hydrochloric solution at a concentration of from about 10% by weight to about 40% weight by weight.

167. The method of Claim 166, wherein the ratio of the weight of hydrochloric acid solution to the source of N-acetylglucosamine as a pure dry weight is from about 1:1 by weight to about 5:1 by weight.

168. The method of Claim 163, wherein the step of hydrolyzing is performed for from about 10 minutes to about 24 hours.

169. The method of Claim 163, comprising the steps of:

- a) hydrolyzing the source of N-acetylglucosamine by combining the source of N-acetylglucosamine with a hydrochloric acid solution or a recycled hydrolysis mother liquor under heat conditions to produce a solution containing glucosamine hydrochloride;
- b) cooling the solution of (a) to precipitate the glucosamine hydrochloride; and
- c) recovering the precipitated glucosamine hydrochloride-containing solids from (b).

170. The method of Claim 169, wherein the step (a) of hydrolyzing is performed by continuously blending the source of N-acetylglucosamine with a hydrochloric acid solution or a recycled hydrolysis mother liquor to maintain the source of N-acetylglucosamine as a dissolved solution, followed by addition of anhydrous hydrochloric acid under heat conditions to the solution of (a) to initiate hydrolysis and convert the N-acetylglucosamine to glucosamine hydrochloride.

171. The method of Claim 169, wherein the hydrolysis mother liquor is hydrolysis solution that remains after recovering the precipitated glucosamine hydrochloride in step (c), wherein a primary or secondary alcohol is added to the hydrolysis solution prior to, during or after a hydrolysis step is performed.

172. The method of Claim 171, wherein the primary or secondary alcohol is selected from the group consisting of: methanol, isopropanol, ethanol, n-propanol, n-butanol and sec-butanol.

173. The method of Claim 169, wherein the step of cooling is performed until the solution is from about -5°C to about 40°C.

174. The method of Claim 169, wherein the step of recovering comprises:

- i) collecting the precipitated glucosamine hydrochloride-containing solids;
- ii) washing the glucosamine hydrochloride-containing solids with a water miscible solvent; and
- iii) drying the glucosamine hydrochloride-containing solids.

175. The method of Claim 169, wherein the step of recovering comprises:

- i) collecting the precipitated glucosamine hydrochloride-containing solids;
- ii) dissolving the solids from (i) in water to form a solution;
- iii) adjusting the pH of the solution of (ii) to between about 2.5 and 4;
- iv) contacting the solution of (iii) with activated carbon to decolorize the glucosamine hydrochloride-containing solids;
- v) removing the activated carbon from the solution of (iv);
- vi) crystallizing glucosamine hydrochloride from the solution of (v).

176. The method of Claim 175, wherein the step of crystallizing comprises concentrating the glucosamine hydrochloride at a temperature of less than about 70°C.

177. The method of Claim 175, wherein the step of crystallizing comprises concentrating the glucosamine hydrochloride at a temperature of less than about 50°C.

178. The method of Claim 175, wherein the step of crystallizing comprises concentrating the glucosamine hydrochloride at less than atmospheric pressure.

179. The method of Claim 175, further comprising recycling solution remaining after the crystallization step (vi) to step (i) of a subsequent recovery process.

180. The method of Claim 175, further comprising recycling solution remaining after the crystallization step (vi) to a subsequent step of crystallization.

181. The method of Claim 175, further comprising washing the crystallized glucosamine hydrochloride from step (vi) with a water miscible solvent.

182. The method of Claim 181, wherein the water miscible solvent is selected from the group consisting of: methanol, isopropanol, ethanol, acetonitrile, acetone, tetrahydrofuran, dimethylsulfoxide, dimethylformamide and dioxane.

183. The method of Claim 181, further comprising drying the crystallized glucosamine hydrochloride after washing at a temperature of less than about 70°C for less than about 6 hours.

184. The method of Claim 183, wherein the step of drying is conducted at less than atmospheric pressure.

185. The method of Claim 183, wherein the step of drying is conducted with an air sweep.

186. The method of Claim 169, wherein the source of N-acetylglucosamine is suspended in an aqueous, low-boiling, primary or secondary alcohol, and wherein the method comprises an additional step, between steps (a) and (b) of removing the acetic acid ester formed with the alcohol following hydrolysis or prior to recycling the hydrolysis solution for reuse.

187. The method of Claim 186, wherein the acetic acid ester is removed by a process selected from the group consisting of: distillation, flashing, and concentration at less than atmospheric pressure.

188. The method of Claim 186, wherein the step of hydrolyzing is performed at a temperature of between about 60°C and about 100°C.

189. The method of Claim 186, wherein the step of hydrolyzing is performed at the solution boiling point at one atmosphere.

190. The method of Claim 169, wherein the step of hydrolyzing is performed at a ratio of hydrochloric acid solution to the source of N-acetylglucosamine as a dry weight of from about 3:1 by weight to about 5:1 by weight, and at a temperature of less than about 80°C.

191. The method of Claim 190, comprising washing the glucosamine hydrochloride recovered in step (c) with a water miscible solvent.

192. The method of Claim 191, wherein the water miscible solvent is selected from the group consisting of: methanol, isopropanol, ethanol, acetonitrile, acetone, tetrahydrofuran, dimethylsulfoxide, dimethylformamide and dioxane.

193. The method of Claim 191, further comprising drying the crystallized glucosamine hydrochloride after washing at a temperature of less than about 70°C for less than about 6 hours.

194. The method of Claim 193, wherein the step of drying is conducted at less than atmospheric pressure.

195. The method of Claim 193, wherein the step of drying is conducted with an air sweep.

196. The method of Claim 157, wherein step (b) of treating comprises contacting the source of N-acetylglucosamine with a deacetylating enzyme to produce the glucosamine product.

197. The method of Claim 196, wherein the deacetylating enzyme is selected from the group consisting of: N-acetylglucosamine-6-P deacetylase and N-acetylglucosamine deacetylase.

198. The method of Claim 196, wherein the deacetylating enzyme is a chitin deacetylase that has been modified to or selected for its ability to deacetylate an N-acetylglucosamine monomer to produce glucosamine.

199. The method of Claim 196, further comprising recovering the glucosamine product by crystallization.

200. The method of Claim 196, further comprising recovering the glucosamine product by precipitation.

201. The method of Claim 196, wherein the deacetylating enzyme is immobilized on a substrate.

202. The method of Claim 196, wherein the step of contacting comprises contacting the source of N-acetylglucosamine with the deacetylating enzyme in the presence of an aqueous sodium or calcium chloride solution.

203. The method of Claim 202, further comprising recovering the glucosamine product by crystallization or precipitation.

204. The method of Claim 196, wherein the step of contacting comprises contacting the source of N-acetylglucosamine with the deacetylating enzyme in the presence of an alcohol to esterify the alcohol.

205. The method of Claim 196, further comprising mixing a salt with the glucosamine product and contacting the mixture with an ion exchange medium.

206. The method of Claim 205, wherein the salt is selected from the group consisting of a chloride salt, a phosphate, a sulfate, an iodide and a bisulfate.

207. A method to produce glucosamine by fermentation, comprising:

a) culturing in a fermentation medium a microorganism which has been transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding glucosamine-6-phosphate synthase, wherein expression of the recombinant nucleic acid molecule is controlled by a lactose induction, and wherein the step of culturing comprises:

5

i) growing the microorganism in the fermentation medium comprising glucose as a carbon source at a pH of from about pH 4.5 to about pH 7 and at a temperature of from about 25°C to about 37°C;

10

ii) inducing transcription of the nucleic acid sequence by addition of lactose to the fermentation medium in the absence of adding additional glucose to the medium;

15

iii) fermenting the microorganism after step (ii) in the presence of glucose at a pH of from about 4.5 to about 6.7 and at a temperature of from about 25°C to about 37°C; and

b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate and glucosamine.

208. The method of Claim 207, wherein a source of trace elements is added to step (iii) of fermenting.

209. The method of Claim 208, wherein the trace elements include iron.

210. The method of Claim 207, wherein step (ii) comprises growing the microorganism in the fermentation medium comprising glucose as a carbon source at a pH of about pH 6.9.

211. The method of Claim 207, wherein step (iii) comprises fermenting the microorganism after step (ii) in the presence of glucose at a pH of from about 4.5 to about 5.

212. The method of Claim 207, wherein step (iii) comprises fermenting the microorganism after step (ii) in the presence of glucose at a pH of about 6.7.